LINEAR INHERITANCE IN TRANSDUCTIONAL CLONES[1]

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THE rule of inheritance in cell lineages is the transmission of an undiminished legacy to each of a geometrically increasing family of descendants. Episodes of mutation or segregation may intervene, but further descendants will again follow this rule of clonal heredity, which is the corollary of equal division. But other rules of inheritance are known—for example, the entailment of estates in land and the traditional law of primogeniture in titles of nobility—whereby a legacy must pass undivided through a single *line* of descent through the generations. This paper will have to do with biological analogies of linear inheritance which have appeared in experiments on the transduction of motility-genes in Salmonella.

Transduction is a mechanism of genetic recombination which is notable for the transfer of hereditary fragments from one cell to another (SYMPOSIUM 1955, LEDERBERG 1956a). In these experiments, a temperate bacteriophage serves as vector for the fragments, which are furnished by the disruption of the chromosomes of a bacterial host as it supports the growth of the phage. When this crop of phage is applied to cells of a suitably marked recipient strain, some ($\sim 10^{-6}$) of these cells yield a transformed clone which carries a given marker from the donor. In previous studies, the transformed clones have exhibited the same genotypic stability as did the parents. However, the selective methods which were used to isolate the rare recombinants would overlook transductional effects that did not yield substantial clones of the new types. These studies included auxotrophic, fermentative, resistance, serological and motility markers, and each one for which a suitable selective technique was available was subject to transduction in much the same fashion.

The following experiments are a follow-up of observations on "motility trails" (see paragraph 1. 1) initiated by DR. BRUCE STOCKER during a research visit to this laboratory (STOCKER, ZINDER and LEDERBERG 1953). After his return to England, DR. STOCKER began microscopic studies on these trails; the immediate concern here was the problem of segregation and crossing over in transductional clones. However, the two studies proved to be operationally inseparable. I am indebted to DR. STOCKER for an unreserved exchange of materials, information and manuscript drafts throughout these studies. In the main, the terminology also follows his suggestions. A concordance of my results and interpretations with his (STOCKER 1956b) is given at the close of this paper.

Glossary and symbols. The central concept of this paper is that of a *line* (adj. *linear* or *unilinear*) which signifies a single, unbranched, finite or infinite chain of descent
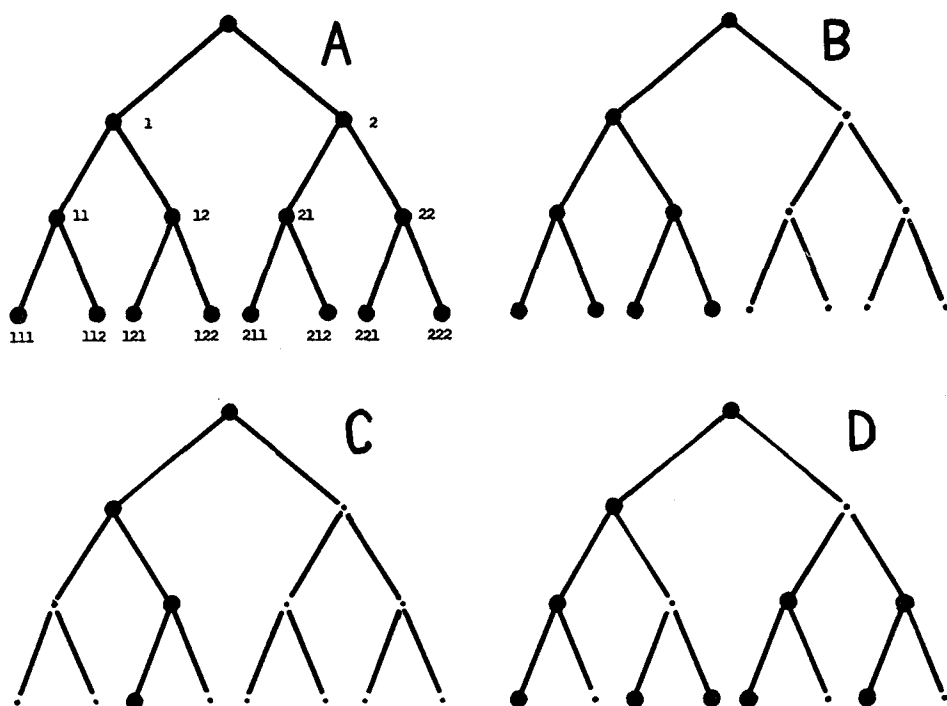
FIGURE 1.—Diagrammatic representations of hereditary transmission within a clone[2]. A, *holo-clone*. B, segregating clone. C, line. D, a pattern of delayed development, see 4.5. The large circles may be taken as $+$, the small as $-$.

(fig. 1C), with regard to a given quality. A line must correspond to a sequence of unequal fissions, at each stage of which only *one* product propagates the line. A clone may be *multilinear* if its descent can be resolved into a number of lines. By extension, a clone or a cell may be loosely referred to as line or linear if it contains or initiates a line. In previous discussions, *semiclone* and *chain* have been used as synonyms of *line*. As will be seen, trails are the overt manifestations of linear inheritance of motility in a clone growing in semisolid agar medium.

Clone is taken to mean the progeny from a single cell, and often implies the regular appearance of a trait throughout that progeny. When the meaning is not given by the context, *holoclone* (see fig. 1A) will be used for the latter sense.

Exogenote signifies a chromosome fragment, explicitly the one given over in transduction. $A — x B$, or its equivalent, $B x—A$, are abbreviations for transduction from genotype A (donor) to B (recipient). These and other terms are discussed in more detail elsewhere (MORSE, LEDERBERG and LEDERBERG 1956b).

[2] A uniform convention for the numbering of cells in a lineage would be helpful. The scheme shown in A is adapted from JENNINGS (1908; cf. ZELLE 1951) and has the advantage that the number of digits is the generation number, and that the family relationships are readily visualized. For very large pedigrees a sequence of *binary* numbers could be replaced by the corresponding *decimal*, but the generation number must then be specified. In C, the line shown is 0-1-12-121. In principle, however, any single line could be written 0-1-11-111-. . . as has been adopted in table 2.

Following the introduction, this paper comprises the following: Section 1 (¶¶1.1 to 1.3) reviews the experimental procedures. Section 2 gives experimental detail on the microscopic analysis of cell pedigrees, and section 3 on plating experiments. Section 4 brings together ancillary observations. The interpretive analysis of the data and speculations are deferred to section 5. Section 6 compares DR. STOCKER's data with these. Many readers may wish to proceed to the recapitulations of the experimental sections, placed for convenience at 2.0 and 3.0, and to the discussions of sections 5 and 6, before reviewing the details.

1. MATERIALS, METHODS AND PRELIMINARY OBSERVATIONS

1.1 General procedures and background observations have been given at length (ZINDER and LEDERBERG 1952; STOCKER et al. 1953; LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956) and will be recapitulated only briefly. In 1897, HISS had discovered that a very soft agar permitted motile bacteria to spread throughout the medium, while nonmotile varieties stayed where put. The technique was redis-covered several times, and has since been widely adopted in enteric bacteriology. The spreading cloudy growth of a motile clone is called a swarm (figs. 7, 8) and is the most characteristic result of transduction of motility to a nonmotile strain (STOCKER et al. 1953). In the same experiments, trails are seen: these are groups of small colonies strung out through the soft agar for some millimeters from the point where the treated bacteria of the nonmotile strain had been planted (fig. 4). The trails were thought to mark the path of a motile cell wherever it divided and left behind nonmotile progeny. The first observations suggested that the trails, and therefore the corresponding lines of inheritance of motility, were always unbranched, but later evidence has weakened this conclusion. At the time, however, the trails were explained by the linear transmission through the recipient clone of a damaged exogenote which could no longer replicate, but could still confer motility on the cell which carried it. Since transduction here fell short of a transformed clone, it was described as abortive. To test the hypothesis of abortive transduction and to supple-ment plating experiments, cell lineages from transformed motile cells were studied more directly in pedigrees controlled by micromanipulation.

1.2 The manipulative procedures, especially the use of the oil chamber, follow DE FONBRUNE (1949). The principal media were Difco penassay broth and NGA, "nutrient gelatin agar", 0.8 per cent gelatin, 0.4 per cent agar with a broth base. Microclones were routinely held at room temperature (22 ± 2°C) and examined at 150 magnifications darkfield. This was conveniently obtained with a 15 × ocular and a 10 × objective (not necessarily phase contrast) in combination with a Bausch and Lomb LWD phase condenser carrying a 43 × annulus. For closer study at 645 ×, a matched 43 × dark phase contrast objective was swung into place.

1.3 Most of the experiments were of the form SW-623 —x SW-666. The cultures are described more fully elsewhere (LEDERBERG and EDWARDS 1953) but both are derived from a monophasic *S. paratyphi B*. SW-666 is Fla_1^- H_1^b (flagellaless, hence nonmotile; phase-1 flagellar determinant b); SW-623 is Fla_1^+ H_1^i. The transducing phage was PLT22 adapted by serial passage on SW-623. SW-666 was originally chosen for these experiments because of the previously demonstrated linkage of the

H_1 to the Fla_1 factor, a consideration which is immaterial except where it is emphasized below.

EXPERIMENTAL RESULTS AND CONCLUSIONS

2. Experiments with microclones

2.0 *Recapitulation.* Partial pedigree analyses were made of the progeny from isolated motile initials. A single initial might generate many motile offspring during the first ten or fifteen generations. When these motile offspring were isolated and propagated, they usually constituted strict hereditary lines (for motility) for as many as thirty fissions longer. In further pedigree analyses, the number of lines which issued from two sister cells was unequally partitioned. That is, if "*E*" represents the potentiality to produce *many* motile offspring, E was unequally, perhaps linearly, transmitted at cell division. Formazan granules were also transmitted linearly, but the possible inherent polarity of the bacteria, as marked by formazan, could not be correlated with the linear transmission of E. About four per cent of motile initials generated stable transductional clones. These often showed an early segregation of motile and nonmotile subclones. The concurrence of motile and multilinear subclones was also noted.

2.1 *Isolation of motile initials.* The first step in each experiment was the isolation of infrequent motile *initial* cells from SW-623 —x SW-666. Equal volumes of an overnight broth culture of SW-666 and stock lysate of SW-623 were mixed and incubated for about 90 minutes to give an input of about 10^9 bacteria, 10^{10} phage per ml. Between 90 and 150 minutes, 10^4 to 10^5 cells became motile. These initials were readily isolated with the help of a trapping droplet. Small drops of the treated cultures were deposited on a cover glass under oil, adjacent to drops of clear broth. The droplets were then fused in pairs, permitting the motile initials to swim into the traps where they could be clearly seen and individually caught. The interval between mixture and isolation was two to three hours, which might allow as many as three fissions depending on a variable initial lag. Subsequent pedigrees are therefore likely to be truncated at the origin. After most of the experiments were completed, it was found that isolation was simplified by spinning down the transductional mixture after about 60 minutes and trapping from droplets of concentrated sediments. Hundreds of initials could be trapped in a short time by this procedure, which was used in experiments below 2.16, 2.18, 3.1, 3.7, 4.1.

2.2 *Undivided clones.* The simplest experiment was to plant the motile initials in individual droplets and examine the microclones the next day, when they usually contained about 10^4 cells each. Table 1 shows the results from 384 viable isolations, including some pedigrees which have been summed to give the total yields. In addition, about ten per cent of the cells isolated were inviable. In these cases, a long filament ("snake") often persisted which might remain motile for hours or days but never divide. Other clones contained one or several ghosts which may have originated by phage lysis.

2.3 Only fifteen (four per cent) of the clones contained a preponderance of motile cells which would relate them to swarms, i.e., holoclonal inheritance of motility These will be taken up later 2.22.

TABLE 1

Distribution of yields of motile cells in 384 single microclones

Yield:	0	1	2	3	4	5	6	7	8	9	10	11–15	16–20	21–30	>30	Swarm
Number of clones:	100	59	39	28	17	7	9	6	6	4	6	25	23	19	21	15

2.4 The remaining clones all had a limited number of motile individuals, ranging with decreasing frequency from 0 to about 100. The distribution of these numbers was highly skewed, as can be seen from table 1. For numbers in the range 0 to 9, the distribution is approximately exponential, each class being proportional to 0.8^x; while the larger numbers follow a nearly flat distribution. Except as an indication of nonhomogeneity, the quantitative significance of this distribution is not apparent, and we shall be concerned only with its qualitative features.

2.5 The microclones therefore give a partial corroboration of the hypothesis of abortive transduction of motility, in so far as transformed motile cells are isolated which do not transmit the trait regularly to their descendants. The frequent class of clones, containing one motile individual is most readily understood: each comprises a line still in being after 13 fissions ($2^{13} \sim 10^4$), while the most frequent, zero class consists of lines that had terminated some time between the first and the 13th fission. Most of the experiments are directed at an understanding of the remaining two thirds of clones which have more than one motile individual: whether these are multilinear, and if so the patterns of distribution or generation of the lines.

2.6 *Progeny of intermediate isolates.* These experiments were done to confirm the linear inheritance of motility for a number of generations, and to ascertain whether the clones containing many motile cells could be resolved into lines, or whether they would show a continuing pattern of irregular transmission which might be neither linear nor clonal. The expression "isolation at n_k" means that a motile individual was reisolated from a clone whose total population indicated a history of k fissions. As indicated in the previous paragraph, many isolates were made at about n_{10} to n_{13}; the history of these pedigrees is summarized in figure 2.

2.7 As shown in figure 2, linear inheritance was followed strictly in every reisolate but one after n_{11}, that is, no branching (production of two motile progeny from one cell) *need* have occurred after n_{11} or *could* have occurred after n_{15}. This range, n_{11} to n_{15}, follows because the clones could not be examined at each fission. Many of the clones were already resolved into one or more lines between the first and tenth fissions. The exceptional clone (*A* in figure 2) must have had a branch not earlier than n_{19} and possibly as late as n_{27}; the figure represents a progeny from a cell isolated at n_{15} which gave a subclone containing 18 motile among 5,000 nonmotile cells. As $18 \sim 2^4$, and $5000 \sim 2^{12}$, this subclone must have branched not earlier than $n = 15 + 4 = 19$, and not later than $n = 15 + 12 = 27$. Ten of the motile cells at n_{27} were isolated; one formed a line for at least 13 additional generations (n_{40}); the others were nonmotile or inviable at the next examination at n_{30} to n_{41}.

2.8 The outstanding examples of continued linear inheritance were a pair of motile cells isolated from the same clone at n_7 which gave regular asymmetric division for
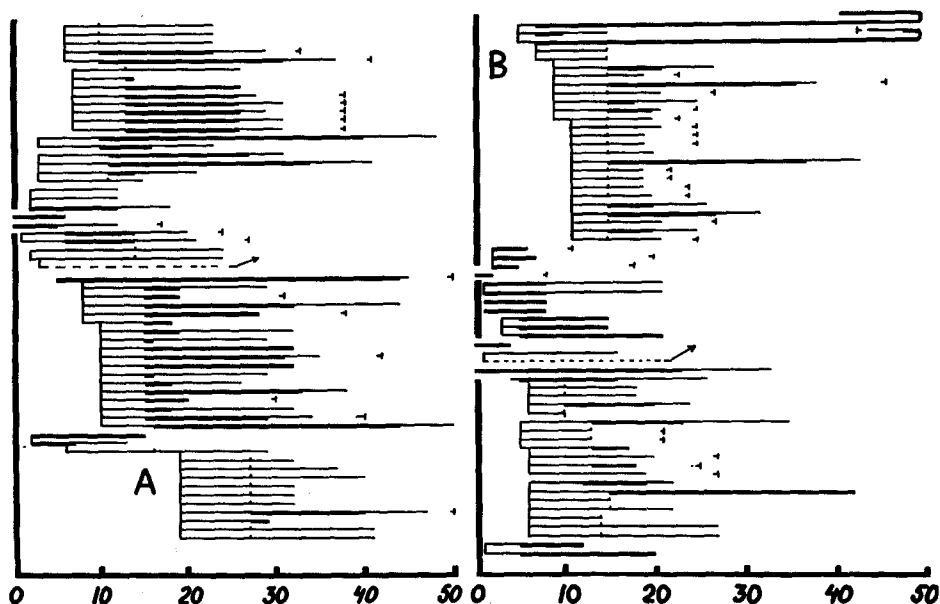
FIGURE 2.—Duration of motile lines generated by isolated initials. The scale is the number of generations elapsed from the time of isolation. The heavy horizontal bars show the minimum duration of a single line, from the last point at which any branching could have occurred to the earliest point at which the line could have terminated. The light bars and extensions show (to the left) the earliest point at which branching could have ceased, and (to the right) the longest that each line might have lasted. The overlap represents intervals during which individual clones were not examined. A vertical tick on a light bar is equivalent to a short heavy bar, that is, a single motile cell was observed and isolated at this point, but had no motile progeny when its subclone was reexamined. Many lines were terminated before motility had ceased; when examinations were continued after the cessation of motility in a line, the end of the study is indicated by ⊣. An arrow indicates a swarm. Vertical bars connect lines that had been isolated from the same clone; they do not represent the derivation of one line from another.

an additional 45 and 52 fissions, respectively (fig. 2B). These lines were observed and reisolated at intervals of a few fissions until they finally terminated, in one case by cessation of growth, in the other by the gradual loss of motility. (This experiment lasted seven days (March 17–23, 1954), the clones being held at 10°C to slow their growth at night.)

2.9 These pedigrees, from intermediate isolates, suggest that clones with many motile cells are indeed multilinear, i.e., can be resolved into simple lines; although branching may persist as late as the third decade of fissions, it is usually not observed after the first. Once established, the lines were propagated for a variable interval (from one to 42 fissions) being terminated either by the death or immotility of the line cell.

2.10 On a number of occasions, a motile line was watched throughout one or more fission intervals. The dividing cell remained active until the moment of separation, at which point one daughter continued to move, while the other remained stationary. The motile daughter was the cell which continued the line on further division.

2.11 *Early lineages from motile initials and partition of line numbers at fission.* It is manifestly impossible to make a complete pedigree analysis of a multilinear clone for more than a few generations, if for no other reason than that one cover glass will not hold more than a few dozen isolates, and that at least a minute is needed, under the best circumstances, to separate two daughters into separate drops. As the clones grew, it became increasingly difficult to complete the handling of a generation before it divided again, and to maintain a coherent record of its disposition. On the other hand, concentration of effort on a single clone was unrewarding because the majority of initials generate none, one or very few lines. No procedure was found (cf. STOCKER, and 4.9) by which the minority of cells that would generate multilinear or swarm-clones could be detected in advance of growing out their progeny. A number of clones were, however, followed for a few fissions to answer the specific question whether the partition of lines (i.e., the total number of motile cells which ultimately appear in sib subclones) was random at the division of a multilinear cell. Figure 3 represents these partitions most of which come from single observations at an early fission. The coordinates of each point give the larger yield as abscissa, the smaller as ordinate. Thus the point (40, 2) refers to a fission at which one daughter gave a clone (usually read at a size of 10^3 to 10^4) containing 40 motile individuals, the other daughter clone giving 2. In a few cases, the partition could be followed for two or three fissions. Only a few of the partitions represent divisions later than n_{10} for the reasons given in 2.7.
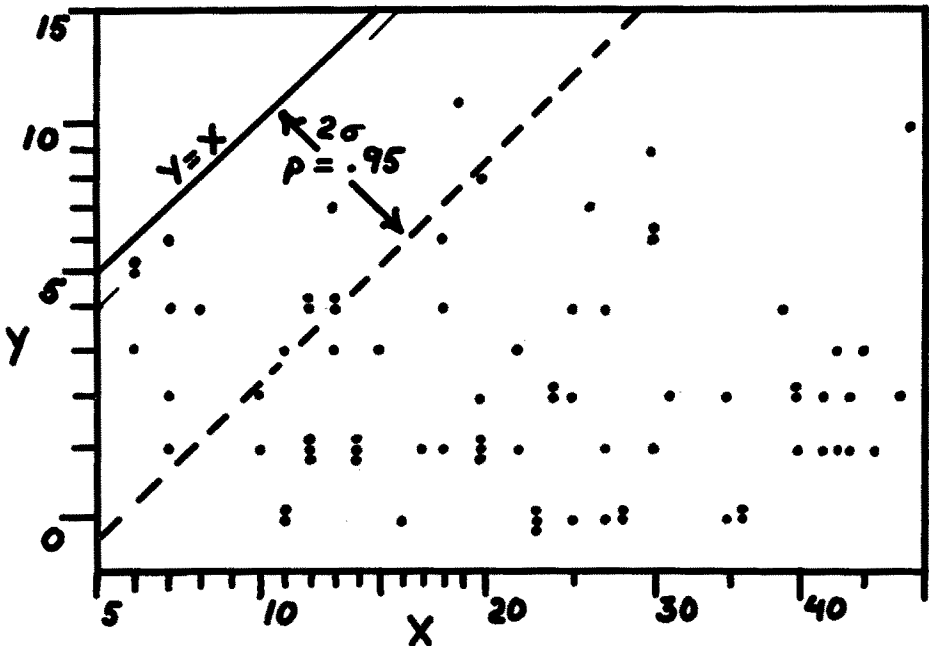

FIGURE 3.—Partition of potential lines at cell division. Each point represents a fission after which the two daughters were allowed to form clones, and the yield of motile lines in each was enumerated. The smaller yield is always given as ordinate. For details of the method of plotting, see 2.12.

2.12 The figure presents only those partitions where a total of eight or more lines was at stake. The data are plotted on a modification of MOSTELLER and TUKEY's (1949) co-ordinate paper. The ordinate scale is shifted to $\sqrt{y + 1}$ rather than $\sqrt{y}$ so that the "shortest distances" can be read directly from the plotted points. The "2σ" band which lies parallel to the expected line, $x = y$, thus should exclude only five percent of the observations. The observed partitions are clearly unequal, one daughter tending to inherit most of the potential lines. The most nearly equal splits of a large stake were 19,11 and 30,9; grossly discrepant splits such as 36,0 or 40,1 were more common.

2.13 These inequalities are consistent with the hypothesis (STOCKER 1956b) that the multilinear clones are resolvable into two orders of linear inheritance, the simple motility lines already described, and in some clones, a line defined by the ability to generate *many* of these simple lines. Following STOCKER, descents with many motile cells will be designated as the "*E* line" (*E* for exceptional), leaving open the question whether the descendancy is strictly linear (see 5.3).

2.14 *Linear inheritance of formazan residue.* Linear inheritance is the expected consequence of the passive handing down of a particle at cell division. An almost trivial instance of a line stems from observations on the bacterial reduction of triphenyl-tetrazolium chloride. The reduction product is triphenylformazan, a fat-soluble, water-insoluble red pigment which is usually deposited as a single conspicuous granule near one pole of the bacterium (LEDERBERG 1948; WEIBULL 1953). The transmission of this granule at cell division has been seen to be linear in direct pedigree isolations, and by other procedures 4.7.

2.15 *Correlation of motility with formazan-lines.* The mere fact of unipolar deposition of formazan speaks for a polarity of cellular organization in Salmonella (and other enteric bacteria) which belies the superficial antero-posterior symmetry of the rod. An effort was made to correlate the unequal division of a cell into formazan-carrying Fz^+ and not-carrying Fz^- daughters, with the unequal division of motility and of E lines. However, it proved to be difficult to induce the deposition of formazan in cells of microclones: in general, it is taken up only by cells in the stationary phase, and it was therefore impractical to study the correlation of formazan with motility in intermediate isolates. Nevertheless, cells already Fz^+ were amenable to transduction, so that pre-stained initials could be isolated. It was however noted that recipient populations, in which half the cells were Fz^+, gave motile initials of which only one to two percent were Fz^+. A similar negative correlation was found between motility and Fz^+ in experiments with motile clones of Salmonella and of *Escherichia coli*. Such clones invariably contain a proportion of temporarily nonmotile individuals which is increased after formazan-staining. However, those motile cells which do carry a unipolar granule appear to be as vigorous and viable as the controls. Possibly the formazan is toxic when it is deposited above a threshold level. No preferred orientation (formazan anterior or posterior) was noted; an individual motile cell might reverse its orientation at intervals of a few seconds.

2.16 Motile initials were collected from transductions to prestained recipients and followed for three or four fissions along the formazan line. That is, at successive fissions, the Fz^+ cell was separated for further observation, and the Fz^- sib set aside

TABLE 2
Yield of motile progeny in pedigree lines of formazan-stained initials

Initial no.	Yield in cell no.			
	2	12	112	Fz = 111
1.	4	25	0	inviable
2.	15	1	2	0
3.	23	0		0
4.	18	0	2	2
5.	13	1	1	2
6.	2	3	44	1
7.	0	22	0	1
8.	swarm	—	0	22
9.	2	18		6
10.	1	2	42	(2)
11.	3	1		12
12.	1	1	0	0
13.	3	0	0	0
14.	1	0	1	0
15.	1	0	1	0
16.	3	0	0	0
17.	3	0	2	1
18.	0	1	1	inviable
19.	2	0	2	0
20.	2	0	0	0

From pre-stained initials, the line of cells (1-11-111) carrying a formazan granule was followed for three fissions, the successive sibs (2-12-112) being transferred to individual droplets. Lines No. 3, 9 and 11 grew more slowly or rapidly than the others, and were therefore not separated at a third fission. Lines 1 to 9 are summarized in 2.16. Line 11 is excluded because of the uncertainty whether 111 or 112 would have been multilinear. In line 10, the granule was no longer discerned after the first fission, and the "formazan-line" was continued only arbitrarily. 12. to 20. are some of the pedigrees with few motile progeny.

In line 8, the initial cell had already divided twice when the isolate was reexamined, and cells 2 (= 21 + 22) and 12 were pooled. Unfortunately, the swarm that resulted was lost before it could be analysed for homogeneity.

The numbering of cells follows figure 1A.

and allowed to form a microclone. Finally, both sibs were set aside, and all the clones were scored for number of motiles the following morning. The sequences of table 2 may be thought of as progressive halvings of the initial cell, from the center to the polar granule. With three fissions, a random disposition would lead to the occurrence of E in successive segments (i.e., 2, 12, 112, 111, see fig. 1A) in the proportions 4:2:1:1. The eight E-clones of this experiment were found in the proportions 4:3:1:0, which is concordant with the expectation, i.e., the E quality is not specifically associated with a part of the initial cell that is marked by Fz polarity. The possibility of mutual exclusion of E and the Fz-marked segment was considered, but another clone (which also contained a swarm 2.22) included a multilinear progeny from the Fz-terminal segment.

2.17 Clones with few lines from the same experiment are also tabulated but show no striking features.

2.18 *Manifestation of linked transduction; tests for reciprocal crossing over.* As already mentioned the Fla^- locus is linked to another marker, H_1, the parental couplings in these experiments usually being $Fla^+H_1{}^i$—x $Fla^-H_1{}^b$. To this point we have considered only the motility phenotype (Fla^+ or Fla^-). The serotype ($H_1{}^i$ or $H_1{}^b$) of motile clones is readily detected with absorbed antiserums by agglutination tests, or by the inhibition of motility in NGA or under the microscope. The Fla—H_1 linkage is exhibited by a proportion of clonal *swarms* which were $Fla^+H_1{}^i$ as well as others which were $Fla^+H_1{}^b$. The serotype of non-clonal initials and *lines* is now in question. The first trials showed a complete inhibition of motile initials equally by b and i serum, and in NGA as well as in microclones. However, control experiments $Fla^+H_1{}^b$—x $Fla^-H_1{}^b$ also showed inhibition by i serum, which must therefore be attributed to a delicate cross-reaction between b and i (not observed in agglutination tests or in inhibition of swarms, and not necessarily a flagellar reaction, cf. LEDERBERG and IINO 1956, ¶3.14) rather than the necessary presence of the i antigen on the motile initials. As a comparable non-b Fla^- stock is not available the specificity of inhibition by b serum has not been verified.

2.19 Other serums (a, c, d, k, r and 1.2) were then tested on the b—x b controls, and all were found non-inhibitory, in contrast to i and b. Further experiments were therefore conducted with the transductions $Fla^+H_1{}^a$ (SW-940)—x $Fla^-H_1{}^b$. With this system, all initials were completely inhibited by 1:1000 b antiserum (figure 11) and were all also partially or completely inhibited by anti-a. In microclones all cells (except a few which generated b swarms) were slowly but completely immobilized by a serum. In dilute NGA + a serum (see 3.3) stationary colonies and a few short trails were seen (fig. 12). This result is evidence, not otherwise available, of the homogeneity of the exogenotes, i.e., that each one that carries the Fla^+ marker also carries the coupled H_1. The occurrence of recombinant clones $Fla^+H_1{}^b$ may therefore be attributed to crossing over in a transient, initial heterogenote $Fla^-H_1{}^b$/ex $Fla^+H_1{}^a$ (cf. MORSE *et al.* 1956b; DEMEREC and DEMEREC 1956).

2.20 Intermediate motile isolates from multilinear clones have also been tested separately with b or a serum. Most were inhibited but in one test, two of four cells isolated were unaffected by a serum, and may have had a pure b serotype. These terminal non-a lines may account for the residual trails seen in NGA platings with this antiserum.

2.21 A search was made for reciprocal crossovers, i.e., $Fla^-H_1{}^a$ among nonmotile progeny in multilinear clones and co-segregants (2.22) in clones containing motile transformations (both $H_1{}^b$ and $H_1{}^a$). These are detected as isolates capable of yielding motile, non-b recombinants when tested x—$Fla^+H_1{}^b$ in NGA plus b antiserum. None were found in a total of about 100 tests.

2.22 *Clones with swarms: segregation.* As stated, 2.3 and table 2, about four percent of motile initials gave clones which contain ten percent or more motile individuals. These have also been replated on NGA and verified to initiate swarms. Several dozen individuals from these progenies have also been allowed to form clones and proved to be regularly motile, without continued segregation. They therefore correspond to stable transductions of motility. However, the initial clones are often mixtures of

stably motile and stably nonmotile cells, shown in terms of proportions of motile to total cells:

Pure +	$\sim\frac{1}{2}$ +	$\sim\frac{1}{4}$ +	$\sim\frac{1}{8}$ +	$<\frac{1}{8}$+
16	8	3	7	2

These data include microclones observed directly as well as those plated in NGA which gave swarms 3.12. The inference that many of the clones are segregating is supported by pedigrees on five additional isolates in which segregation was observed directly until not less than n_1 (two cases), n_2 (two cases) and n_4 (one case). One of the n_1 pedigrees, already cited in 2.16 generated twenty-two lines in a cosegregant of the swarm. Only two other pedigrees (which segregated swarms to at least n_1 and n_2 respectively) were directly informative on the number of lines generated by co-segregants (non-swarm sibs) of swarm-producing cells. The numbers were zero and one respectively (cf. 3.12).

3. *Plating experiments*

3.0 *Recapitulation.* Motile initials and single clones therefrom were plated in soft agar, and the correlation of motile individuals and lines with trails was verified. However, the fraction of motile cells which formed trails in agar depended on the stiffness of the medium, and no generalization could be made on the distinction of linear from multilinear cells by this method. The general features of the clones, already stated from pedigree experiments, were reaffirmed in the plating experiments. Several trails might be generated by a single initial. The early segregation of motile and nonmotile holoclones was verified, as was the occurrence of motile holoclones (swarms) as sibs to multilinear subclones.

3.1 *Correlation of motile initials with trails in NGA.* It has so far been plausibly assumed that motile lines correspond to visible trails of colonies in NGA. However, experiments to verify this correspondence were at first indifferently successful. Motile initials were pooled after isolation from trapping droplets (2.1) and transferred individually to fresh drops. These were then taken up, one at a time, in a hand-controlled quartz pipette (LEDERBERG 1954) and blown out on to the surface of an NGA plate. By this technique of blind transfer (which is, however, much less laborious than the more certain procedure of transfer by the micropipette used for manipulation) somewhat more than half the isolations were successful in terms of outgrowth at the point of transfer. Thus, 29 motile initials planted on NGA gave 18 outgrowths: three were swarms, the remainder were stationary colonies or clusters of two or three fused colonies. No trails were seen.

3.2 As it was supposed that chemotactic influences (see 4.11) might encourage motile bacteria to remain at the surface layer of the agar, trials were made of plating motile initials in deep NGA. Pools of initials were collected and ejected from the micropipette into 0.5 ml broth. Samples of the diluted pool were than made up to contain 100 or 200 cells and plated with 10 ml molten NGA in 6 cm Petri dishes, or 25 ml NGA in 10 cm Petri dishes. The plates were then chilled to set them promptly

and incubated overnight at 37°C. All too often, the readings were already somewhat obscured by overspreading swarms, but it was usually possible to enumerate swarm centers as well as colonies and trails. As an arbitrary classification, a *trail* is an aggregate of ten or more microcolonies in NGA; a *cluster* has from two to ten; a *colony* (self-evidently) just one, and a *swarm* is a diffuse cloud of growth. These units, taken together, are *viable centers*. The summation of a number of platings from several preparations in which about 900 initials had been plated is:

	Colonies	Clusters and trails	Swarms	Viable centers
Number.....................	732	86	31	849
Per cent....................	86	10	4	

3.3 This incidence of swarms is comparable to the yield in table 1; it is quite clear that only a small proportion (here ten percent) of the motile cells plated can form trails in NGA. However, considerable variability in the proportion of trails was noted from one day to the next. This variability was traced to the fluidity of the NGA, which is poorly reproducible unless special care is taken to disperse all of the gelatin and agar evenly. It was found that the addition of progressive volumes of broth, to dilute the NGA, would give progressively higher yields of trails, while concentrating the medium had the opposite effect. For example, aliquots of the same pool of initials were plated, with the following results.

NGA diluted with broth	Colonies	Clusters	Trails	Swarms	Viable centers
—	53	2	2	2	59
4:1	46	5	6	0	57
7:3	39	18	11	3	71
3:2	19	11	19	4	53

Clearly cells that appear only as compact colonies in NGA (fig. 7) are manifest as clusters and trails when the medium is diluted (fig. 8). The last medium (dilute NGA) was adopted as the softest that could be cleanly handled without slopping (except in midsummer).

3.4 The platings of well-separated initials show "trails" which are no longer strikingly linear, especially in dilute NGA. At least part of the rectilinearity of the trails formerly figured may be attributable to the orientation of a chemotactic gradient of metabolic products from the excess recipient cells (cf. 4.11). The isolated trails may more closely resemble the tracings of random walks but their own appearance, together with that of seeded NGA under the microscope suggests that the progressive movement of motile cells in this medium is confined to microscopic interstices in the gel. These interstices may also entrap motile cells, which would account for the appreciable proportion of initials which give no trail at all; even when NGA is seeded with fully motile clones, only a minority of the cells are visibly motile at any one time.

3.5 The rectilinearity of trails is, of course, also complicated by the genetic multi-
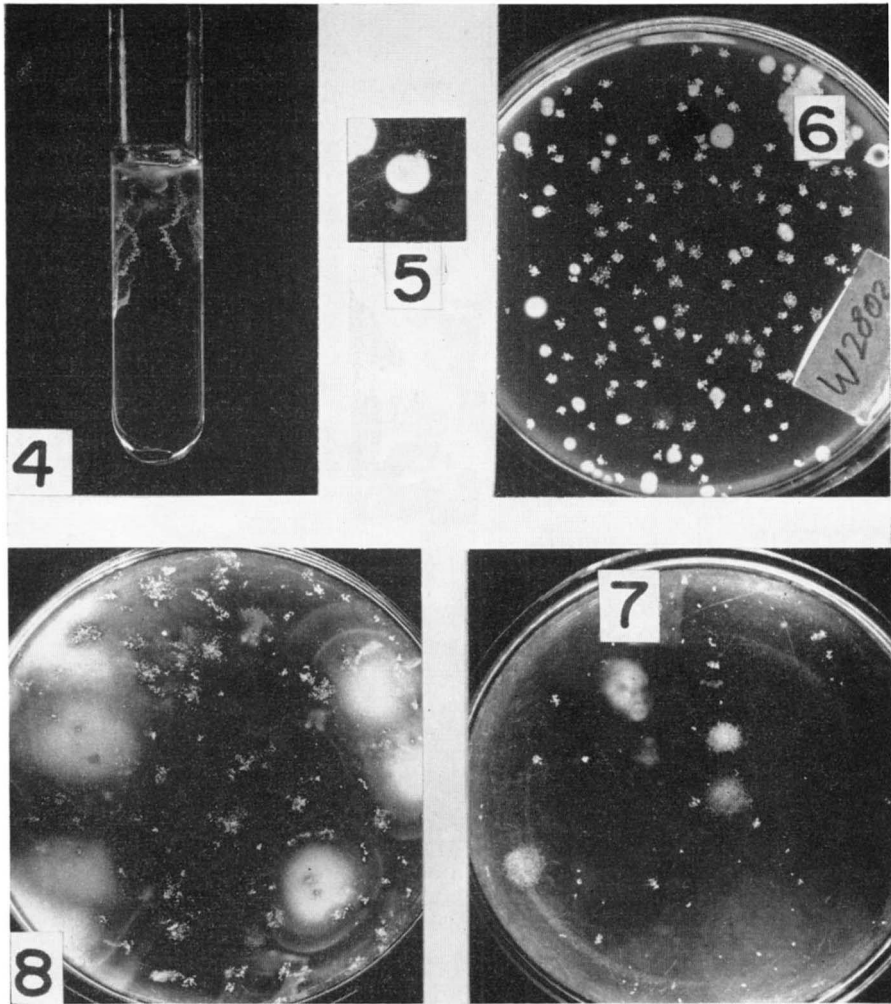
FIGURE 4.—Unusually long trails from SW-553 x— SW-666. The transduction mixture was diluted and samples planted at the top of a series of NGA tubes. Many of the tubes showed swarms, which appear as cylindrical clouds which gradually progress to the bottom, as well as trails. This tube lacks swarms but shows two trails of unusual length. The tube was incubated several days before being preserved for photography, and some irrelevant growth at the glass-agar interface is also evident. This and other figures approximately life size unless indicated otherwise.

FIGURE 5.—A colony of SW-553 in deep NGA showing a spontaneous trail. About 3×.

FIGURE 6.—E. coli mutant W-2802 showing abundant satellites, in deep NGA.

FIGURES 7–8.—Pooled initials from SW-666 x— in NGA and dilute NGA respectively.

linearity of the clones produced by many initials. A few platings have been made of intermediate motile cells, isolated ca. n_{10}. The trails these cells produce are generally less profuse than those made by initials and they may sometimes assume a definitely rectilinear aspect, though the presence of doublets is again remarkable.

3.6 The occurrence of swarms in these platings has also been noted. Owing to
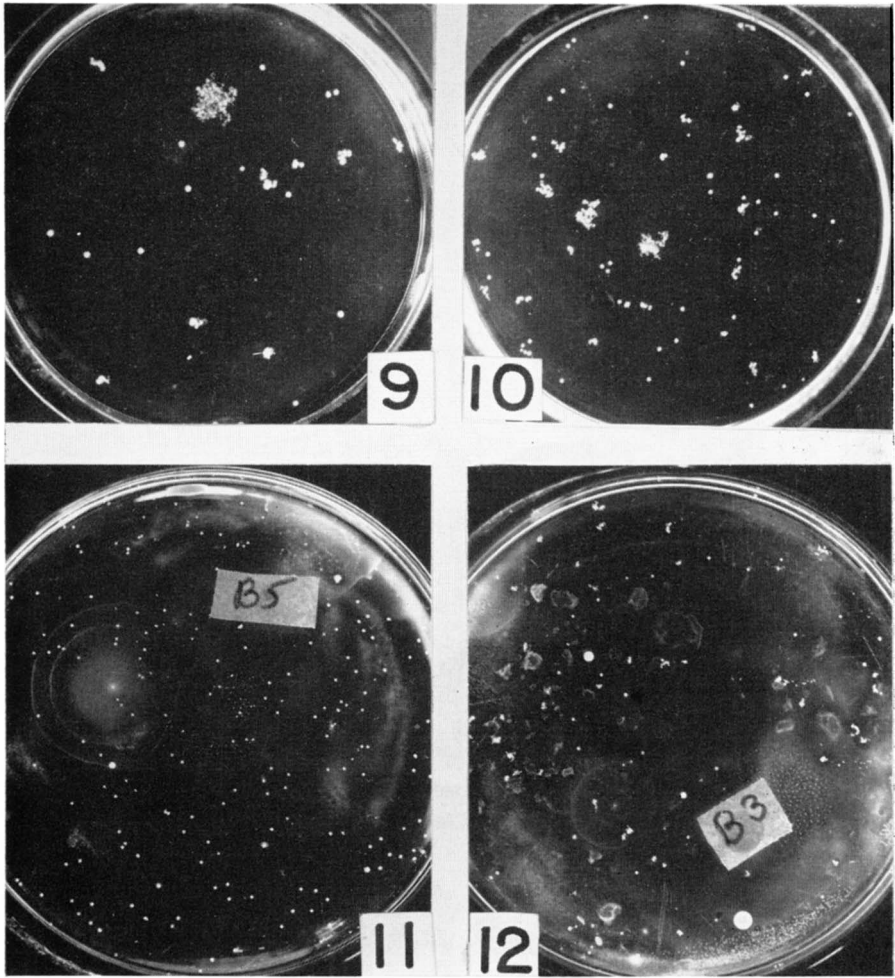
FIGURES 9–10.—Single clones of SW-666 x— from motile initials showing several large and small trails each. Plated in dilute NGA.

FIGURES 11–12.—Pooled initials of SW-666 x— SW-940 in dilute NGA containing b and a antiserum, respectively. Compare with figure 8.

overgrowth, observation of swarm centers has been difficult, but in favorable plates, about half the swarms are notably centered by a compact colony or a trail, indicative of early segregation as the counterpart of 2.21. This centering was not observed when motile clones were replated, and should not be confused with "flares" 4.6.

3.7 *Platings of clones.* The technique of 3.1 was used to transfer single initials to .5 ml volumes of broth. These were then incubated about three hours, and plated into NGA. The plates were then incubated overnight. Altogether, 282 isolates were transferred, 186 successfully. The clones averaged about 2^6 viable centers each, but showed considerable variation, presumably because of a high dispersion of the duration of lag (cf. CAVALLI and LEDERBERG 1956). For example, the following clone

sizes were noted in one series of parallel platings: 7, 8, 10, 17, 21, 23, 25, 30, 32, 44, 44, 54, 65, 66, 69, 73, 81, 87, 101, 106, 109, 140, 142, 143, 149. The non-integral powers of 2 also illustrate early dissynchrony, though this may be exaggerated by the temporary staying together of essentially divided cells. About five percent of the viable cells are not recovered in the plates, as was shown by counts in deep agar added to the residue in the broth tubes after plating. These variables indicate the need for caution in postulating statistical homogeneity in bacterial growth.

3.8 Of the 186 viable clones that were plated, 155 showed only compact single colonies, or occasional doublets of doubtful significance, precisely as did platings of clones of control SW-666 cells. Sixteen clones included one trail, three clones had two and one (possibly in a soft batch of NGA) had ten trails in addition to compact colonies. The three or four exceptional clones indicate that the potentiality of forming a trail even in NGA is not a strictly unilinear legacy (cf. 5.3).

3.9 The remaining 11 clones included swarms which usually spread so that they could not be counted. Three of these were pure; 8 had colonies or trails as well, and are therefore included as examples of segregation in 2.22.

3.10 After the effect of diluted NGA was discovered (3.3), 62 initials were processed for the plating of clones in this medium. As expected, the incidence of trails was much higher than in the preceding experiment. Thirty-nine viable clones included 22 with trails (see figs. 9, 10), 15 with single compact colonies only, and two with swarms, both segregating. The following numbers of motile lines (trails and clusters) were found in this series of clones: fourteen zeros, and 1, 1, 1, 2, 3, 3, 4, 6, 8, 8, 9, 11, 11, 11, 14, 15, 17, 20, 20, 21, 21, 25. As compared with table 1 this distribution may be shifted slightly to the right, which is expected because motile lines that terminate before n_{10} would be missed in microclones, but may well be detected in dilute NGA.

3.11 A few clones were also processed from intermediate isolates. In accord with 2.6 these gave at most one trail.

3.12 In five of the thirteen swarm-clones of these experiments, the numbers of cosegregant trails could be counted for comparison with 2.21 as follows: 2, 3, 5, 18, and 20. The other five segregating swarm-clones either had compact colonies or, if trails were present they were obscured by the swarm. As already noted, three of the swarm clones were pure.

4. Miscellaneous observations

4.1 *Experiments with SW-553; spontaneous lines.* SW-553 is, as previously described (LEDERBERG and EDWARDS 1953) a nonmotile *Salmonella dublin*, $Fla^-H_1^{gp}$. Unfortunately, many experiments which later had to be repeated with SW-666 were first conducted with SW-553. This stock was initially chosen because its Fla^- mutation, although distinct from that of SW-666 (STOCKER *et al.* 1953) is also linked to H_1. Furthermore it gives spectacularly long trails (fig. 4) when motility is transduced to it from other strains. However, it has also been found to produce trails spontaneously, though these are always insignificant when compared to those obtained by transduction. This effect was first noticed in platings of clones from motile initials (cf. 3.7) when every colony in some plates was observed to have a small

satellite trail (as in fig. 5). The same effect was then observed in control platings. It can be discerned regularly only in dilute NGA, which accounts for its having been overlooked previously. SW-666 did not give this effect in comparable platings, and was therefore adopted in place of SW-553 for further studies. Otherwise, the general features of the transduction experiments with SW-553 were quite similar to those reported in 2. and 3.

4.2 When it was learned how to trap motile initials from concentrated suspensions (2.1), the procedure was applied to untreated cultures of SW-553, and motile initials were found about one thousandth as frequently as from transductional mixtures. These were isolated and found to generate lines which petered out after a few divisions, which, together with the appearance of their minute trails in agar makes them comparable to the late lines in transductional clones (2.7).

4.3 Other *Fla⁻* cultures which produce spontaneous trails more frequently have been noted (STOCKER *et al.* 1953) and are being studied more systematically (QUADLING, cited by STOCKER 1956a).

4.4 A similar appearance, with more profuse satellites, has been noted in a number of mutants which were recovered in so far unsuccessful attempts to obtain absolute nonmotile mutants of *E. coli* K-12 (fig. 6); the patterns of transmission of motility in this material have not been investigated in detail but are seemingly quite irregular. In general, *E. coli* is not as aggressively motile as are most Salmonellas.

4.5 *Delay of expression in motile clones.* When cells from motile clones of Salmonella were observed at fission, both daughters were promptly and equally motile. Salmonella is, however, characterized by numerous flagella per cell, so that this result is not inconsistent with a latent polarity. LEIFSON (1951) has described bacteria with unipolar flagellation in two categories, based on his observations of stained smears: those in which incipiently dividing cells already show a flagellum at each (distal) pole, and those in which only one flagellum is apparent. The second situation would be attributed to a relative delay in the formation of a new flagellum after the onset of fission. Through DR. LEIFSON's courtesy, a culture of this type (*Pseudomonas aeruginosa* H1A) was available and preliminary observations on living material have been made. The most extensive pedigree is depicted in figure 1D, where + and − refer to apparently motility of a cell at the moment of its separation from its sib. Thus, every cell transmits motility to its progeny. However, a + initial has one +, one − daughter; a − initial two + daughters. This rule can be rationalized if a flagellate cell makes no more flagella, while its daughter, *née sans* flagella, makes two *de novo* prior to the next fission. As is also perhaps to be expected, occasional exceptions to this rule were found in other more fragmentary pedigrees, and more work will be needed to validate the pattern on a sound statistical basis.

4.6 *"Flares"*. STOCKER *et al.* (1953) noted that the centers of transductional swarms often contained an accumulation of denser microcolonies, very variable in size. At that time, it was wondered whether these might represent a segregational process, but no nonmotile derivatives could be isolated from these "flares" (the actual center of the swarms being crowded with excess recipient cells). It is now apparent that the flares have no special relationship to the transduction process, as they have been seen on replatings of motile clones, both of transductional and stock culture origin.

They are especially prominent in platings of rough Salmonella strains and of motile *E. coli*, and are presumably related to autoagglutination. Selection for rapid motility in motile clones, or of the smoothest colony forms in nonmotile recipients, minimizes the flares which are produced on direct plating, or in transduction experiments, respectively.

4.7 *Applications of viscous adjuvants (Methocel)*. Through the courtesy of The Dow-Corning Company, Midlands, Michigan, Methocel (methylated cellulose) preparations of various viscosity types were available for these experiments. Methocel 4000 (the viscosity in cps of a two percent aqueous solution) made up in broth and kept in microdroplets in an oil chamber was found to keep the daughter cells together after bacterial fission, so that intact chains of 2, 4, 8, to as high as 128 and 256 cells are formed. These are useful as a simple means of detecting certain relationships in cell lineages. For example, cells with a polar formazan granule generate chains in which the granule is still regularly observed at a polar position in the terminal cell. A few percent of the stained cells carried the granule interstitially and correspondingly generate chains with the granule in an interstitial cell (usually at a polar position). The formazan granule is thus inherited linearly, each division along the line giving one Fz^+ and one Fz^- cell. The formazan is evidently metabolized by Samonella cells, as the granule gradually fades and disappears in the course of five to ten fissions.

4.8 The regularity of these chains makes them sensitive indices of liminal amounts of multiplication and death, which are especially difficult to detect if they are concurrent (cf. discussion by Ryan 1955). Multiplication is revealed by the increment of two- and four-celled chains in an initially dispersed suspension, and death by gaps (in which faint ghosts can sometimes be made out) in the chains. Chains allowed to develop for three to six fissions and then held for a day or two in the refrigerator were found to suffer about one percent of deaths; however these were not correlated with each other, with formazan granules or either end of the chain, and are therefore considered as indeterminate events, rather than consequences of "aging" or genetic damage.

4.9 After preliminary trials, Methocel 400, one and one-half percent in broth was found to give a threshold level of viscosity that barely permitted most of the cells of a motile clone (from a previous pedigree isolation, and not selected for augmented motility) to continue moving. Large pools of initials were placed in droplets of this medium, but no initial cells were found whose motility (during the first three hours after the transduction mixture was made up) approximated that of the motile clone. Later, motile swarms overgrew the drops as expected. Clearly the full development of the motility phenotype of a Fla^+ clone requires considerably longer than its first manifestation. About one percent of the initial cells gradually crept into the methocel in an hour's time, but these cells did not consistently generate a higher fraction of swarms or multilinear clones than unselected samples of initials. These experiments were hindered by an undue wetting of the cover-glasses by the methocel solutions, so that the drops eventually spread and mixed with one another. Attempts to discriminate future swarm- or multilinear clones by the maximum persistence of activity of the initials for one or two fission intervals in ordinary broth were equally inconclusive.

4.10 Trials were made on the incorporation of methocel to regulate the viscosity of agar, but flocculent precipitates and poorly gelling media resulted.

4.11 *Chemotaxis and bacterial behavior.* There is an extensive literature on chemotactic behavior in bacteria but as most of it is fifty or more years old (cf. JENNINGS 1906) it is no longer often quoted. As controls on experiments 2.19 the agglutination of motile clones by homologous antisera was followed microscopically. Paired droplets of bacteria and serum (1:100 and 1:1000) were arranged on a cover-glass over an oil chamber in preparation for mixing. After several drops had stood for about ten minutes it was noticed that the motile bacteria had accumulated at the distal portion of their drops, leaving the proximal portion almost free of cells. The two drops were separated by several mm. of oil, and the accumulation of cells was readily seen by the naked eye. The experiment was readily and reproducibly repeated, but not so readily understood. However, it was found that the negative taxis did not depend on the specificity of the serum, but that only preparations issued by the Central Public Health Laboratories, Public Health Laboratory Service, London, England, showed the effect. It was then realized that these preparations were preserved with five-tenths percent cresol, and this substance (or phenol in its stead) was then inculpated as the tactic stimulus. Minute amounts of phenol (from a source which may be only 10^{-5} M to start) evidently can dissolve and diffuse through the mineral oil to the bacterial drop and establish a concentration gradient in which the bacteria seek the lowest level. The behavioral basis for the migration (cf. JENNINGS 1906; ROTHSCHILD 1956) is not obvious, but is more likely to depend on comparisons at points along the path of the cells, rather than on a continuous vectorial orientation of the cells along the chemical gradient, as no such orientation was observed. At the sites of accumulation, the cells showed very rapid but jerky movements (i.e. a short mean free path) as compared to their initial behavior and this may account for the accumulation, without offering a clue as to how the chemical information (at such low concentrations) is transmuted into behavioral response (JENNINGS and CROSBY 1901). The levels of phenol employed here are quite innocuous to the viability of the organisms. The phenol effect could not be obtained with *E. coli* nor, (it goes without saying) with nonmotile strains. Attempts to quantitate the response have been hindered by its failure to occur, at least in such spectacular fashion, in capillary tubes where concentration gradients and rates of migration could be accurately controlled and measured. A somewhat similar behavioral response is evoked by staled medium (regardless of pH). In trapping procedures, motile initials have been noticed to accumulate to some degree in the zone at the margin between the mass culture and the empty drop, rather than occupy the latter freely, which is a slight hindrance to fishing for them with a micropipette.

4.12 *Position effect?* At least five distinct *Fla⁻* mutations each linked to H_1 have been recorded (STOCKER *et al.* 1953; LEDERBERG and IINO 1956). The following four have been tested in all transductional combinations: SW-666, SL-28; SW-553 and SW-1157. (The fifth, SL-13, poses certain technical difficulties as it responds very meagerly to the phage and cannot be used as donor.) In every combination, not only swarms but also trails were observed, which indicates that these mutants though commonly linked and affecting the same character do not show a cis-trans position effect (cf. 5.6 and MORSE *et al.* 1956b).

5. DISCUSSION

5.1 Three principal topics will be discussed: evidence for and material interpretation of linear inheritance in Salmonella, analogous examples of asymmetric cell division, and their significance for inferences of biological particles. Because more than one interpretation can be put on the data of this paper, they have been left as bare as possible up to this point. Two aspects of linear heredity are in question: the linear propagation of motility from intermediate isolations, in which linear inheritance is directly observed, and the possibly linear propagation of "*E*" which can be inferred as indicated below, 5.3.

5.2 *Motility lines.* These require little discussion as extensive pedigree 2.7 and some plating analyses 3.5, 3.10, 3.11 leave little doubt of uncomplicated, linear transmission of motility in many *sub*clones.

5.3 *Linear partition in multilinear clones.* Contrary to first expectation STOCKER *et al.* 1953) motile initials often generate more than one motile line. The quality *E*, of generating many motile lines, is partitioned very unequally at fission (fig. 3). Does *E* constitute a line? The distribution (table 1) suggests that about ten lines might bound "few" from "many", and only one point on figure 3 (perhaps a statistical overlap) shows both sibs to have generated more than ten lines each. However, *E* has not often been followed in pedigrees beyond the first few divisions, so that the duration and singularity of the postulated *E* line would not have strong evidential support. Furthermore, several clones contained both *E* and motile subclones. With these reservations (see also 6.2) the present experiments are indecisive but not grossly incompatible with the hypothesis that *E* is linearly inherited.

5.4 *Material equivalents; particles and lines.* Linear inheritance conveys a compelling suggestion of a discrete, nonreproducing particle which is passively inherited by one offspring of a dividing cell. It is, furthermore, plausible to correlate the motile line with a motility-conferring particle, a flagellum or its Anlage, a blepharoplast. There is no direct evidence for this identification, which might be helped by electron-microscopic descriptions of unilinear cells as uniflagellate. However, for the sake of further argument this hypothesis will be tentatively adopted. Nevertheless it should be cautioned that the immediate basis of linear inheritance is recurrent, asymmetrical division, and that this can be accomplished in ways in relation to which the hypothetical particle would be a remote abstraction.

5.5 The correspondence of *E* with a particle is, for its part, independent of whether its transmission is strictly or almost linear, as the laws of distribution of the particle can readily be accommodated to fit either experimental result. Many of the experiments in this paper are therefore, from this point of view, directed to a secondary issue. The following hypotheses are stated for the strict linear case; the qualifications needed to suit them for occasional deviations are obvious.

5.6 The termini of these experiments are fairly clear: we begin with a transduced exogenote and end up with a flagellum. We can therefore ask two questions of *E*, which are not necessarily logically related: what is the relationship of *E* to each of these elements. To give hypothetical answers for the latter first, we may postulate:

A. *E* is a bundle of flagella, or of units bearing a unitary relationship to them. *E* therefore divides (but does not multiply) in a requisite asymmetrical pattern.

B. E is a catalyst or precursor of flagellar formation. (These alternatives cannot be distinguished without stoichiometric data on flagellar synthesis.)

Some answers to the other question are:

1. E is the exogenote which has failed to exchange with the recipient chromosome in a reproductively efficient fashion and therefore fails to reproduce. Its present location, and the nature of the accident that led to the abortion are unspecified.

2. E is "products of gene action". These may have accompanied the exogenote in transduction, or may have been synthesized in a transient heterogenote when the exogenote first inhabited the recipient cell. They result in motile lines when they are left in cells from which the exogenote has disappeared or segregated.

The combination of (A,B) with (1,2) leads to four alternative, tenable versions of which the following models are suggested as plausible representatives:

A1. A polytenic exogenote is transduced and, failing to be incorporated, is sorted out among the progeny of the initial cell. Each unitary chromonemal segment makes (or is converted into) one flagellum. The bundle of chromonemata tends to splinter rather than divide equally at cell division.

A2. An exogenote is transduced and begins to function in the recipient cell regardless of the success of incorporation. Consequently numerous flagella are formed at an active pole of the cell. The exogenote may then disappear, or segregate into a sib cell. The flagella are no longer synthesized, but are asymmetrically sorted out among the progeny, thus generating a multilinear clone.

B1. An exogenote is transduced and, failing to be incorporated, persists as a functional, nonreproductive particle. It therefore mediates the formation of flagella in the line of cells which carries it. The flagella are sorted in the progeny of the progressive sib clones.

B2. An exogenote is transduced and mediates the formation of a flagellum-synthesizing system or organelle. This system is transmitted linearly and continues to function after the exogenote is lost from the cell which carries it.

5.7 For exceptions to the unilinearity of E, we can simply postulate that the exogenote (under 1) rarely multiplies or (if initially ditenic) divides; under 2, the exceptions would reflect the imperfection of polarized division of the cell.

5.8 The data of the present paper do not permit a critical choice among these alternatives. For A versus B we should need more knowledge of the chemical and morphological pathways from gene to flagellum. For 1 versus 2, a conceivable experiment may be possible with increasing knowledge of the linkage map of Salmonella, and the availability of two genetically linked but physiologically unrelated markers that could be diagnosed in linear inheritance. If the markers remained linked in lines, we would either have to question their physiological unrelatedness or admit their association in lines on a genetic basis, which would be tantamount to 1, the hypothesis of the the residual exogenote.

5.9 The experiments on the persistence of $H_1{}^a$ action and 2.18 may be considered as models of the proposal, but this marker is too closely related in its action to Fla to make a convincing argument. Thus, the persistence of the a antigen may mean no more than that an antigenically differentiated intermediate has been formed in a cell which initially carried $H_1{}^a$ as well as $H_1{}^b$.

5.10 Finally more knowledge of bacterial growth may bear on the plausibility of asymmetrical fission via a system other than a discrete particle. Meanwhile, we may have to admit that this material gives us no means of differentiating the action of a gene from that of its intermediaries, as long as both are so hypothetical.

5.11 *Segregation of swarm-clones.* The fact that swarm clones are often mixed with Fla^- 2.22 might be hoped to throw some light on the mechanism of incorporation of transduced exogenotes. However, it has an almost trivial explanation in the multi-nucleate character of enterobacterial cells, and similar early segregation has been recorded in experiments on mutational (LEDERBERG 1949; WITKIN 1951; NEWCOMBE 1953; RYAN and WAINWRIGHT 1954) sexual-segregational (LEDERBERG 1956b), lyso-genizing (LEDERBERG and LEDERBERG 1953; LIEB 1953) and other transductive (MORSE et al. 1956a) systems. The variation in extent of segregation may also depend on the truncation of early pedigrees 2.1 and on a variable delay before incorporation occurs. These variables make it impossible to lay any weight on segregation as evidence for the copy-choice versus direct replacement hypothesis of transductive incorporation (SYMPOSIUM 1955, HOTCHKISS 1956). It has already been emphasized that incorporation of a marker involves the displacement of its homologue, not merely an addition to the genotype, as can be shown particularly with antigenic markers.

5.12 In the transduction of *Gal* loci in *E. coli* (MORSE et al. 1956b) segregation can be delayed indefinitely. That is, a heterogenote is formed in which the fragment can proliferate coordinately with the cell, before segregation occurs sporadically. It is therefore readily shown that incorporation involves crossing over, in the sense that a variable part of a given exogenote appears in the progeny of different segregation events. In Salmonella, the heterogenote initially formed by transduction evidently cannot persist and incorporation and segregation of the exogenote must follow promptly on the injection of the phage. It has previously been noted (STOCKER et al. 1953, and is now corroborated in the material of 2.22) that no clones contained two serotypes of swarms, which is a further argument for the transiency of the hetero-genote stage. However, the uniform coupling of Fla^+ with $H_1{}^a$ in motile initials 2.19, and their separation in $Fla^+H_1{}^b$ swarms support a cross-over model of incorporation, similar to that which is more directly inferred for *E. coli*.

5.13 *Asymmetry in cell division: bacteria.* Enteric bacteria appear to be simple rods, lacking head-tail asymmetry. Other bacterial species are more clearly differentiated, spectacularly in Caulobacter which has a long stalk, terminated by flagella at one end (HOUWINK 1955). At cell division, the body of the cell is split, concurrently with the development of a flagellum and ultimately a stalk at the distal (free) pole. These observations also put in question the possibility of genetic continuity of the flagellar Anlage, (BISSET 1956) unlike the division processes described in many protozoa. Bacteria with unipolar flagellation, like those studied by LEIFSON (1951) pose the same problem of the morphogenetic relationship of the old and new flagella. At any rate, each fission of such a cell may be considered to engender one new cell (in respect to the flagellum) and leave one old one. Whether the sequence of oldest cells in a series of fissions will constitute a detectable line can be thought of as depending on how completely a mature flagellar apparatus is resynthesized by the young cell in an inter-

fission interval. The motility lines would represent the extreme case where no such synthesis is possible owing to genetic defect; intermediate situations are represented by spontaneous trails 4.1, and by the observations on *Pseudomonas aeruginosa* 4.5 and *E. coli* 4.4.

5.14 The overt symmetry of Salmonella and other bacillary species may then be only superficial; suggestions of head-tail or corresponding mother-daughter differences come from the unipolar deposition of formazan 2.14 and the basophilia of one or both poles, interpreted by BISSET (1956) as indicative of a growing point. Differences in the reaction of sib daughter cells to staining procedures (PENNINGTON 1950) and to antibiotics (LINZ 1954; SMILES, WELCH and ELFORD 1948) are also indicative of unequal division. However, these scattered observations have not yet been correlated to the extent that this conception of the organization of the bacterial cell may be regarded as more than a speculative working hypothesis.

5.15 *Protozoa.* Outside the bacteria and simpler algae, very few unicellular organisms divide by simple fission, so that there are many opportunities for asymmetric division and linear transmission. For example, JENNINGS (1908) and McCLENDON (1909) have described the linear inheritance of accidental pellicular defects for as many as twenty-two fissions. This pattern is a simple consequence of the conservation of the surface structure of the Paramecium at cell division. In a related experiment, JENNINGS (1937) injured the teeth of Difflugia by surgery and found that both daughters of an injured individual were often defective. The deviation from linear transmission in this case is apparently due to the role of the teeth of the mother cell as models for the deposition of the daughter's. In an appropriate context, then, silica can simulate a gene. However, successive offspring from the line of cells carrying the original defect were progressively more normal. The experimental defect therefore did not generate a defective clone, but one with a variety of defective lines. In all of JENNINGS' experiments, the morphological description of the propagation of variations averts the postulation of discrete particles.

5.16 *Algae.* The algae furnish several examples of lines which depend on peculiarities of cell division. A desmid, for example consists of two modified hemispheres, "semi-cells" joined by a narrow isthmus in which the single diploid nucleus is lodged. At cell division, the nucleus undergoes mitosis and the semi-cells separate, each one later budding a new semicell to form a new cell. WARIS (1950) and KALLIO (1951) have described mutations in Micrasterias which seemingly have a cytoplasmic basis, since the progeny of a defective semi-cell remain defective, and of the normal, normal, despite the common nuclear origin. In one case, a reversion was described which resulted in a cell with one normal, one mutant semi-cell. This "dichotypic" initial generated a clone in which all of the new semi-cells thenceforth were normal. The clone was thus unilinear for dichotypy: the equivalent "particle" in this instance may be the whole cytoplasmic architecture of the differentiated, mutant semi-cell which persists after the genotype is altered by mutation.

5.17 The growth of diatoms also generates lines of a sort. The cell is bounded by two half-shells which fit into one another like the lid and base of a Petri dish. In many species, the walls are so rigid that they limit the growth of the daughter cells after division. Therefore, the daughter that receives the *inner* half-shell of the parent.

and uses this for its *outer* half-shell, is regularly smaller than the other daughter, which maintains the same size as the parent. Each clone therefore contains only one line of cells as large as the parent, and a series of lines of progressively smaller size. The mean cell size of a clone diminishes progressively with time, but can be restored by the formation of (often sexual) auxospores in which the rigid walls are discarded. As the cells become smaller they lose the capacity for auxospore formation and ultimately for vegetative division too, and the clones are therefore doomed (WIEDLING 1948; cf. RIZET 1953). A formal scheme of progressively attenuated particles might have been constructed for this finite longevity, but again is less meaningful than the inference from direct observation of the division mechanism.

5.18 *Yeast.* Two types of lineation are possibly related to the proliferation of yeast by budding. The disproportionate partition of total cytoplasm may account for the lines of normal mother cells, with small-colony-variant buds, described by EPHRUSSI and HOTTINGUER (1951) in experiments with acriflavine. BAUTZ and MARQUARDT (1954) have indicated that anaerobic, Nadi-negative mother cells placed in air remain negative but form positive-buds. On the other hand, SPIEGELMAN (1951) concluded that the cytoplasm was equally divided between mother and bud from an analysis of another cytoplasmic character. If both observations are correct, it may be necessary to assume that acriflavine actively influences the segregation of the postulated plasmids, perhaps by aggregating them.

5.19 A second effect of budding is to leave a "birth scar" on the bud, and add a bud scar to the wall of the mother. Each clone should therefore continue a line of the oldest cell, carrying the most scars. Since a new bud was never observed to form at the site of an old scar, the oldest line, indeed any line, presumably has a finite lifetime, (BARTON 1950, BARTHOLOMEW and MITTWER 1953), though this might be up to one hundred buddings; twenty-three were observed by BARTON. Remarkably, BARTHOLOMEW and MITTWER reported having found one cell with twenty scars: its incidence in the entire clone would be calculated at 2^{-20} or one in four million.

5.20 *Morphogenesis.* Morphogenetic differentiation must always be related to unequal division in the long run. When divergent clones are produced, the process resembles segregation; however, stem cells of various kinds (e.g. apical cells in shoot meristems; teloblasts in annelid embryos) form approximations to lines, although the detailed cell linages have not often been worked out. In the renewal of spermatogonia in the rat, however, CLERMONT and LEBLOND (1953) have secured evidence that the ancestral spermatogonia undergo cycles of fission whereby one daughter (or son?) proliferates and differentiates into eight or sixteen spermatocytes, and the other remains an undifferentiated stem cell. The material foundation of the line of stem cells is unknown.

5.21 *Orthoclones and senescence.* The line of individuals of a given age in a series of progenies has been designated an "orthoclone" by LANSING (1948) who has also described progressive changes in old orthoclones of rotifers.

5.22 The relationship of linear inheritance to aging mechanisms may be conceived in terms of individual cells of the line (or orthoclone) or to the average properties of the clone. Thus, SONNEBORN (1930) demonstrated that the line of head-bearing individuals on repeated fission of the worm Stenostomum has a finite life time, while

the line of tail segments can be propagated indefinitely. The aging of a head may be related to the concentration of differentiated structures at that end of the worm; tail segments regenerate new (i.e. "young") heads at fission. Attempts to demonstrate a similar aging process in bacterial cells 4.8 and in yeast 5.19 have been unsuccessful, but have been carried for only a limited number of generations. The maternal ortho-clone in yeast should ultimately show the effect of progressive scarring.

5.23 On the other hand, if we define aging as the progressive loss of a function in a clone, we should consider the progressive dilution of the parental character as any uni- or multi-linear clone increases. Thus an initially motile individual of Salmonella generates a clone in which the average motility tends toward zero, and reaches it with the occasional termination of lines. The aging of a diatom clone is a parallel but more obvious example. In these examples, however, the parental line is itself the most juvenile element of the population, in so far as it most closely resembles the initial state.

5.24 *Chromosomes*. Recent studies on the material (in contrast to the informational) content of chromosomes and of the deoxyribonucleic acid of phage have raised the question whether mitosis is ever equal. The Watson-Crick model of DNA structure (CRICK 1954) postulates two complementary polynucleotide helices, which might separate at mitosis and generate the alternative complements. Recent experiments by LEVINTHAL (1956) are consistent with this semi-conservative model of replication of phage DNA: after the initial separation, each helix is conserved as such and the material content of the original DNA will be found as two lines in a holoclone carry-ing its genetic information. Analogous studies by MAZIA and PLAUT (1955) on Crepis chromosomes indicate a single line, as does the suggestion of tinctorial differences between "mother and daughter chromosomes" (PROKOFIEVA-BELGOVSKAIA 1946; cf. MULLER's criticism appended thereto). It is therefore plausible to look for linearly transmitted modifications that have a chromosomal basis.

5.25 *Particles*. The axiomatic foundation of genetic analysis is the particle. How-ever, the term should not be taken too literally, especially when applied to a mathe-matical rather than a material entity. Particulate forms of inheritance apply to such amorphous items as acreage, titles and protoplasmic lumps, from which we can infer that a *particle* is essentially a rule of division, which can be stated in physical, legal, or biological terms, and whose domain of validity must not be ignored (cf. LEDER-BERG and LEDERBERG 1956).

6. CONCORDANCE WITH STOCKER (1956b)

6.1 No irreconcilable differences obtain between the two studies, which differ pri-marily in the strains used, and in the emphasis given to the interpretation of the linearity of E.

6.2 In his material, STOCKER found a greater discontinuity in the distribution of lines per initial than I show in table 1, and his is flatter for the lower yields. We are in full agreement on the disproportionate partitions of figure 3. He has found six pedigrees (for my one, 2.7) in which an E cell was recovered late in the clone. His pedigree analysis of the linearity of E is more detailed, but it is impossible for it to

be complete 2.11 and the main evidence for this comes from the partition data. I have already commented on possible rare exceptions to E-linearity 5.3. These might be misclassifications due to the overlap of E and non-E distributions, or experimental errors, rather than valid exceptions. On the other hand they are sufficiently rare that STOCKER's data and mine cannot be shown to be statistically heterogeneous. Finally, we have used slightly different materials and, perhaps, techniques.

6.3 It has not been possible to make a direct test of the assertion that only an E-cell will form a trail in NGA. We agree that the incidence of trail-forming and of multilinear initials is about the same. The progressive increase in yield of trails as the NGA is diluted 3.3 suggests that this agreement in "standard NGA" is fortuitous, and the stochastic factors (such as entrapment by agar fibrils) are decisive in trail formation. The occurrence of three or four (out of 186) clones with more than one trail, 3.8, argues against either the linearity of E, or the one to one correspondence of trails with E cells.

6.4 I have tried to show 5.5 that the exact linearity of E is not crucial for its material interpretations. STOCKER's preference for 5.6 B1, the residual exogenote, is entirely plausible as a working hypothesis, but the alternative versions cannot be discounted at present.

SUMMARY

1. A hereditary *line* is an unbranched chain of descent, based on recurrent unequal division. Two aspects of linear inheritance were observed in studies of transduction in Salmonella: the persistent linear transmission of motility itself, and the (approximately) linear transmission of the trait "E", *viz.* the generation of *many* motile *lines.*

2. Initial motile cells were isolated from transductions of motility to a nonmotile mutant, and the progeny from these initials studied microscopically and in soft agar plates. Four percent of the initial cells gave stable motile recombinants, analogous to the transformations of other traits previously studied. The remaining cells gave descents which contained one or more motile lines. Many of these lines were studied to verify that a motile cell would regularly divide to give one motile, one non-motile offspring.

3. The motile lines are considered to arise from a non-dividing phenotypic residue, perhaps a flagellum, from a previous "abortive transduction" of a motility gene.

4. Some of the clones from motile initials had many (ten to forty) motile lines. The partition of potential lines at cell division was regularly unequal. Thus it was concluded that E is also inherited in linear or nearly linear fashion.

5. A number of hypotheses for this behavior were considered, notably that either a sterile (non-replicating) chromosome fragment or an intermediate product (phenotypic residue) of its action is responsible. Methods of distinguishing these hypotheses are discussed, but none are presently available for this material.

6. Various aspects of transduction mechanics (including early segregation in transformed clones, range of fragment size, position effect) are reviewed. Conceptual parallels for linear inheritance in genetics, development and ageing, and the semantics of "particle" are speculatively discussed.

LITERATURE CITED

BARTHOLOMEW, J. W., and T. MITTWER, 1953 Demonstration of yeast bud scars with the electron microscope. J. Bacteriol. **65:** 272–280.

BARTON, A. A., 1950 Some aspects of cell division in *Saccharomyces cerevisiae.* J. Gen. Microbiol. **4:** 84–86.

BAUTZ, E., and H. MARQUARDT, 1954 Sprunghafte Änderungen des Verhaltens der Mitochondrien von Hefezellen gegenüber dem Nadi Reagens. Naturwiss. **41:** 121–122.

BISSET, K. A., 1956 Cellular organization in bacteria. Symposia Soc. Gen. Microbiol. **6:** 1–18.

CAVALLI-SFORZA, L. L., and J. LEDERBERG, 1956 Isolation of pre-adaptive mutants in bacteria by sib selection. Genetics **41:** 367–381.

CLERMONT, Y., and C. P. LEBLOND, 1953 Renewal of spermatogonia in the rat. Am. J. Anat. **93:** 475–501.

CRICK, F. H. C., 1954 The structure of the hereditary material. Sci. American **151:** 54–61.

DE FONBRUNE, P., 1949 *La Technique de Micromanipulation.* Paris: Masson Cie.

DEMEREC, M., and Z. E. DEMEREC, 1956 Analysis of linkage relationships in Salmonella by transduction techniques. Brookhaven Symposia in Biology **8:** 75–84.

EPHRUSSI, B., and H. HOTTINGUER, 1951 On an unstable cell state in yeast. Cold Spring Harbor Symposia Quant. Biol. **16:** 75–85.

HISS, P. H., 1897 On a method of isolating and identifying *Bacillus typhosus.* J. Exptl. Med. **2:** 677–700.

HOTCHKISS, R. D., 1956 The genetic organization of the deoxyribonucleate units functioning in bacterial transformations. In *Enzymes, Units of Biological Structure and Function.* Edited by O. H. GAEBLER. New York: Academic Press.

HOUWINK, A. L., 1955 Caulobacter, its morphogenesis, taxonomy and parasitism. Antonie van Leeuwenhoek **21:** 49–64.

JENNINGS, H. S., 1906 *Behavior of the Lower Organisms.* New York: Columbia Univ. Press.
   1908 Heredity, variation and evolution in protozoa. J. Exptl. Zool. **5:** 577–632.
   1937 Formation, inheritance and variation of the teeth in *Difflugia corona.* J. Exptl. Zool. **77:** 287–336.

JENNINGS, H. S., and J. H. CROSBY, 1901 The mechanism by which bacteria react to stimuli, especially to chemical stimuli. Am. J. Physiol. **6:** 31–37.

KALLIO, P., 1951 The significance of nuclear quantity in the genus Micrasterias. Ann. Bot., Soc. Zool. Bot. Fenn. **24:** 1–122.

LANSING, A. I., 1948 Evidence for aging as a consequence of growth cessation. Proc. Natl. Acad. Sci. U. S. **34:** 304–310.

LEDERBERG, E. M., and J. LEDERBERG, 1953 Genetic studies of lysogenicity in *Escherichia coli.* Genetics **38:** 51–64.

LEDERBERG, J., 1948 Detection of fermentative variants with tetrazolium. J. Bacteriol. **56:** 695.
   1949 Bacterial variation. Ann. Rev. Microbiol. **3:** 1–22.
   1954 A simple method for isolating single microbes. J. Bacteriol. **68:** 258–259.
   1956a Genetic transduction. Am. Scientist **44:** 264–280.
   1956b Conjugal pairing in *Escherichia coli.* J. Bacteriol. **71:** 497–498.

LEDERBERG, J., and P. R. EDWARDS, 1953 Serotypic recombination in Salmonella. J. Immunol. **71:** 232–240.

LEDERBERG, J., and T. IINO, 1956 Phase variation in Salmonella. Genetics **41:** 743–757.

LEDERBERG, J., and E. M. LEDERBERG, 1956 Infection and heredity. Symposia Soc. Developm. Growth **14:** 101–124.

LEIFSON, E., 1951 Staining, shape, and arrangement of bacterial flagella. J. Bacteriol. **62:** 377–389.

LEVINTHAL, C., 1955 The mechanism of DNA replication and genetic recombination in phage. Rend. Ist. Lombardo Sci. **89:** 192–199.

LIEB, M., 1953 The establishment of lysogenicity in *Escherichia coli.* J. Bacteriol. **65:** 642–651.

LINZ, R., 1954 La multiplication de *Mycobacterium tuberculosis* en présence de streptomycine. Ann. Inst. Pasteur **86:** 334–346.

MAZIA, D., and W. PLAUT, 1955 The distribution of parental material in chromosome reproduction. Biol. Bull. **109:** 335–336.

McCLENDON, J. F., 1909 Protozoan studies. J. Exptl. Zool. **6:** 265–283.

MORSE, M. L., E. M. LEDERBERG, and J. LEDERBERG, 1956a Transduction in *Escherichia coli* K-12. Genetics **41:** 142–156.

1956b Transductional heterogenotes in *Escherichia coli*. Genetics **41:** 758–779.

MOSTELLER, F., and J. W. TUKEY, 1949 The uses and usefulness of binomial probability paper, J. Am. Stat. Assn. **44:** 174–212.

NEWCOMBE, H. B., 1953 The delayed appearance of radiation-induced genetic change in bacteria. Genetics **38:** 134–151.

PENNINGTON, D., 1950 A reaction of bacterial cells with formaldehyde. J. Bacteriol. **59:** 617–623.

PROKOFIEVA-BELGOVSKAIA, A. A., 1946 "Mother" and "daughter" chromosomes. J. Heredity **37:** 239–246.

RIZET, G., 1953 Sur l'impossibilité d'obtenir la multiplication végétative ininterrompue et illimitée de l'Ascomycète *Podospora anserina*. Compt. Rend. **237:** 838–840.

ROTHSCHILD, 1956 *Fertilization*. London: Methuen.

RYAN, F. J., 1955 Spontaneous mutation in non-dividing bacteria. Genetics **40:** 726–738.

RYAN, F. J., and L. K. WAINWRIGHT, 1955 Nuclear segregation and the growth of clones of spontaneous mutants of bacteria. J. Gen. Microbiol. **11:** 364–379.

SMILES, J., F. V. WELCH, and W. J. ELFORD, 1948 The influence of antibacterial substances on the interaction of bacteria and bacteriophage. II. J. Gen. Microbiol. **2:** 220–227.

SONNEBORN, T. M., 1930 Genetic studies on *Stenostomum incaudatum*. J. Exptl. Zool. **57:** 57–108.

SPIEGELMAN, S., 1951 The particulate transmission of enzyme-forming capacity in yeast. Cold Spring Harbor Symposia Quant. Biol. **16:** 87–98.

STOCKER, B. A. D., 1956a Bacterial flagella. Symposia Soc. Gen. Microbiol. **6:** 19–40.

1956b Abortive transduction of motility in Salmonella; a non-replicated "gene" transmitted through many generations to a single descendant. J. Gen. Microbiol. **15:** 575–598.

STOCKER, B. A. D., N. D. ZINDER, and J. LEDERBERG, 1953 Transduction of flagellar characters in Salmonella. J. Gen. Microbiol. **9:** 410–433.

Symposium on Genetic Recombination, 1955 J. Cell. Comp. Physiol., **45:** Suppl. 2.

WARIS, H., 1950 Cytophysiological studies on Micrasterias. II. The cytoplasmic framework and its mutation. Physiol. Plantarum **3:** 236–246.

WEIBULL, C., 1953 Observations on the staining of *Bacillus megaterium* with triphenyltetrazolium. J. Bacteriol. **66:** 137–139.

WIEDLING, S., 1948 Beitrage zur Kenntnis der vegetative Vermehrung bei Diatomeen. Botan. Notiser: 322–354.

WITKIN, E. M., 1951 Nuclear segregation and the delayed appearance of induced mutants in *Escherichia coli*. Cold Spring Harbor Symposia Quant. Biol. **16:** 357–372.

ZELLE, M. R., 1951 A simple single-cell method for genetic studies of bacteria. J. Bacteriol. **61:** 345–349.

ZINDER, N. D., and J. LEDERBERG, 1952 Genetic exchange in Salmonella. J. Bacteriol. **64:** 679–699.